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Melia dubia leaf fodder has higher volatile phytochemicals during winter: A case study across Satpura and northern Western Ghats Mountain ranges, India

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ABSTRACT: *Gas-chromatography mass-spectrometry (GC-MS) analysis of M. dubia leaf elucidated variation in volatile chemical compounds across provenances and seasons. In total, an array of 93 volatile phytochemical compounds was revealed. Winter season leaf samples contained maximum (55) compounds as compared to summer (35). Corroboration of detected chemical compounds with available literature divulged that similar compounds occur in other species of same and other families as well and have one or the other beneficial biological activities. The presence of volatile compounds in M. dubia leaf could be a rich source antioxidant, anti-inflammatory, and antibacterial activity etc. And leaf feeding to small ruminants and livestock may have many biological benefits in terms of favored growth performance, production quality, and enhanced endogenous antioxidant systems.*

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1. INTRODUCTION

Tree leaf fodder contains a variety of secondary metabolites, including antioxidant peptides, phenols, flavonoids, phytic acid, trypsin inhibitors, and lectins (Diaz-Sanchez *et al.*, 2015; Dey *et al.*, 2019; Wong et al., 2020; Sukhadiya *et al.*, 2021). These plant-derived secondary metabolites are rich in antioxidant, anti-inflammatory, and antibacterial activity (Diaz-Sanchez *et al.*, 2015, Kumar and Goel, 2019). Phytochemicals have been reported to have positive antioxidant benefits toward animals in terms of favored growth performance, production quality, and enhanced endogenous antioxidant systems, possibly by directly affecting specific molecular targets or/and indirectly as stabilized conjugates affecting the metabolic pathways (Hamaker *et al.*, 2009; Jorgensen *et al.*, 2010; Wong *et al.*, 2020). Accordingly, dissecting the antioxidant effects and the underlying mechanism of dietary phytochemicals is an important area.

Currently, cultivation of fodders is limited to only 4.00% of the total cultivable land in the country. The performance of livestock and the economics of milk production are profoundly dependent on the quantity and quality of fodder fed. There is a current shortfall of 35.60% green fodder, 10.50% dry fodder, and 44.00% concentrate feed across the country. The inadequacy of nutrients is a major limitation for livestock as a deficit of 728 mT (64%) of green fodder and 157 mT (25%) of dry fodder with 27% of crude protein (CP) and 24% of total digestible nutrients in India (Singh *et al.*, 2022). This can partly be overcome by feeding tree leaves and other organs, as huge quantity of biomass is available from fodder trees, which provide nitrogen, energy, minerals and vitamins and reduce cost of feeding (Wong et al., 2020; Sukhadiya *et al.*, 2022). Tree foliage and other organs (drupes, pods) are important component of livestock diets and plays important role in the nutrition of herbivores in areas where few or no alternatives are available (Kamalak *et al.*, 2010; Sukhadiya *et al.*, 2019). Therefore, taking cue from the importance of these volatile and non-volatile chemical compounds, we intended to analyze these in *Melia dubia* Cav. leaf fodder. It shed leaves in December and new flush of leaves appear in February-March along with the flowers. The species is indigenous to the Western Ghats region in India and is common in moist deciduous forests of the Indian states from Kerala to Gujarat and also found in Bangladesh, Myanmar, Thailand, Mexico, Sri Lanka, Malaysia,

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Indonesia, China, Philippines, Australia and African countries (Thakur *et al.*, 2021; Sukhadiya *et al.*, 2021). It is a large deciduous tree of Meliaceae family, much advocated multipurpose industrially important (Parthiban *et al.*, 2019; Thakur *et al.*, 2023) and acceptable agroforestry species and gaining importance in wood-based industry (Mohanty *et al.*, 2019; Thakur *et al.*, 2018; Chavan *et al.*, 2022). Hence, looking in to need to explore new species as to feed source present study was undertaken to ascertain its leaf volatile compounds.

2. MATERIAL AND METHODS

Provenances and geo-climatic conditions

Eight provenances [two (P_1 & P_2) falling in Satpura mountain ranges and six (P_3 & P_8) in northern most tip of western Ghats or Sahyadri mountain ranges] were selected in Gujarat, India (Table 1). Sagai-1 and Sagai-2, provenances area characterized by black cotton soils, with average (30 years) annual normal rainfall of 924.8 mm and average annual maximum and minimum temperature of 34.7°C and 20.7°C, respectively. Nanaponda and Kaprada provenances, a northern extension of Central Indian Deccan Plateau, belong to late Cretaceous-early Eocene age and followed by Quaternary sediments, have black cotton soils. The maximum daily temperature ranges from 32.2°C to 41.2°C while minimum temperature from 9.9°C to 23.3°C. Average annual rainfall in these provenances is 2399 mm and 2842 mm, respectively. Waghai and Chichinagavtha, and Mahal and Ahwa provenances fall under south and north Dangs region, respectively. These areas have black rock-outcrops, shallow black, brown and alluvial soils of recent origin. The average annual rainfall ranges from 1500 to 3000 mm and average annual temperature is about 26°C and maximum temperature may shoot up to 46ºC and the winter temperatures 22°C.

Sample collection and processing

Five trees (each tree representing one replication) were selected randomly from each provenance for leaf fodder sample collection. Tree growth parameters *viz.* tree height and diameter at breast height (DBH) and average canopy area (m^2) were recorded (Table 1). Leaf samples were collected from all directions (north-south and east-west) at lower, middle and upper tree canopy. The samples were washed with distilled water in laboratory and air-dried followed by drying in oven at 60°C±5°C till constant weight was achieved. The dried samples were then finely grounded (particle size<1 mm) using grinder and laboratory grade pulverizer. Volatile and non-volatile compounds were estimated for winter and summer seasons of 2020 and 2021 and averaged to ascertain the provenance and seasonal variations.

Volatile phytochemical estimation through Gas-chromatography mass-spectrometry

M. dubia leaf sample phytochemicals were detected through Gas-chromatography mass-spectrometry (GC-MS) following Murugesan *et al.* (2013).

3. RESULTS AND DISCUSSION

M. dubia leaf volatile phytochemicals

Non targeted gas-chromatography mass-spectrometry (GC-MS) analysis of *M. dubia* leaf samples of different provenances and seasons *i.e.* winter (S_1) and summer (S_2) seasons, revealed an array of 93 volatile phytochemicals (Table 2). Chromatograms (randomly selected 4 out of 16 chromatograms of 8 provenances and two seasons) showing peak area and retention time of detected phytochemicals are depicted in figure 1 to 4. Total 76 and 60 compounds were detected in winter and summer, respectively (Table 3). Maximum of 55 and 35 compounds in winter and summer sample, respectively, were detected from Sagai-2 (P_2) provenance. The lowest (23) compounds were detected in Chichinagavtha provenance (P_6) in winter and summer. As many as, 43 common phytochemicals (Table 3) numbered 5, 7, 9, 13, 17, 19, 20, 23, 24, 26, 27, 30, 35, 36, 40, 45, 46, 47, 49, 52, 53, 55, 57, 60, 61, 62, 63, 64, 65, 69, 70, 73, 74, 76,77, 80, 81, 82, 85, 88, 89, 92 and 93 were identified in winter and summer

Table 1. Geographical location of *M. dubia* provenances (P_1-P_2 in Satpura mountain ranges and P_3 -P_8 in The Northern most Western Ghats or Sahyadri mountain ranges) in Gujarat India, selected in the study

Provenance (P)	Geographical attributes			Average biometric attributes		
	Altitude (m)	Longitude (E)	Latitude (N)	Tree height (m)	DBH (cm)	Canopy area (m^2)
Sagai-1 (P1)	373.68	73°47'33.13"	21°40'19.28"	25.80	155.73	246.00
Sagai-2 (P2)	320.04	73°44'21.30"	21°40'47.32"	35.90	172.00	256.16
Nana Pondha (P3)	63.09	73° 8'54.14"	20°26'5.45"	26.60	128.20	206.20
Kaprada (P4)	382.52	73°15'23.07"	20°20'15.71"	25.22	120.18	241.40
Waghai (P5)	127.41	73°29'4.63"	20°45'49.32"	33.76	156.99	221.20
Chichinagavtha (P6)	256.03	73°33'40.46"	20°47'19.06"	30.64	148.40	188.50
Mahal (P7)	262.13	73°36'6.52"	20°57'0.13"	42.52	149.00	157.40
Ahwa (P8)	319.13	73°39'24.02"	20°46'1.27"	36.60	117.20	126.60

Table 2. ***M. dubia*** **leaf volatile phytochemicals detected using non targeted gas chromatography mass spectrometry (GC-MS)**

S.No.	Compound	Compound chemical formula & Molecular weight
1	(E)-Labda-8(17), 12-diene-15,16-dial	$C_{20}H_{30}O_2$ (302.45)
2	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$ (278.34)
3	1,3-Dioxolane, 2-butyl-2-methyl-	$C_8H_{16}O_2$ (144.21)
4	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(	$C_{30}H_{50}O$ (426.7)
5	10-Heneicosene (c,t)	$C_{21}H_{42}$ (294.55)
6	1-Cyclohexyldimethyl silyloxy-3,5-dimethylbenzene	$C_{16}H_{26}$Osi (262.46)
7	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$ (242.44)
8	1-Decanol, 2-octyl-	$C_{18}H_{38}O$ (270.5)
9	1-Dodecanol, 2-hexyl-	$C_{18}H_{38}O$ (270.49)
10	1-Dodecanol, 2-octyl-	$C_{20}H_{42}O$ (298.55)
11	1-Heneicosanol	$C_{21}H_{44}O$ (312.57)
12	1-Heptacosanol	$C_{27}H_{56}O$ (396.73)
13	1-Hexadecanol	$C_{16}H_{34}O$ (242.44)
14	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-	$C_{20}H_{40}O$ (296.53)
15	1-Methyl-3-n-propyl-2-pyrazolin-5-one	$C_7H_{12}N_2O$ (140.18)
16	1-Undecanol	$C_{11}H_{24}O$ (172.31)
17	2,3-Dimethyl-3-pyrazolin-5-one	$C_5H_8N_2O$ (112.13)
18	2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10, 10a-octahydro-5, 5a-dihydroxy-4-(hy	$C_{30}H_{46}O_6$ (594.31)
19	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$ (206.32)
20	2-Dimethyl(ethenyl) silyloxytetradecane	$C_{19}H_{40}$OSi (344.7)
21	2-Dimethyl(ethenyl) silyloxytridecane	$C_{13}H_{27}$Osi (312.6)
22	2-Oxazolamine, 4,5-dimethyl-	$C_5H_8N_2O$ (274.37)
23	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$ (268.47)
24	2-Propenoic acid, 2-(acetylamino)-	$C_5H_7NO_3$ (129.11)
25	2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol mesylate	$C_{17}H_{18}$-C_lN_3O (315.79)
26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$ (296.53)
27	3-Octenoic acid, 4-methyl-2-pentyl ester	$C_{14}H_{26}O_2$ (226.35)
28	4a,7a-Epoxy-5H-cyclopenta[a]cyclopropa [f]cycloundecen-4(1H)-one, 1a,6,7,1	$C_{28}H_{40}O_{12}$ (568.6)
29	4aa,4bß-Gibbane-1a, 10ß-dicarboxylic acid, 4a-formyl-2ß,7-dihydroxy-1-meth	$C_{22}H_{30}O_7$ (406.5)
30	4-Heptanol, 2, 6-dimethyl-4-(1-methylethyl)-	$C_{12}H_{26}O$ (186.33)
31	5,5,6-Trimethylhept-3-en-2-one	$C_{10}H_{18}O$ (156.26)
32	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$ (296.48)
33	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$ (276.37)
34	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$ (254.40)
35	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{19}H_{32}O_2$ (278.42)
36	9-Nonadecene	$C_{19}H_{38}$ (266.50)
37	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$ (396.48)
38	Acetic acid, 3,7,11,15- tetramethyl-hexadecyl ester	$C_{22}H_{44}O_2$ (340.58)
39	Antiquorin	$C_{20}H_{28}O_3$ (316.43)
40	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$ (390.55)
41	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$ (368.59)
42	Cholesta-8,24-dien-3-ol, (3ß,5a)-	$C_{27}H_{44}O$ (284.63)
43	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$ (296.48)
44	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$ (426.68)
45	dl-a-Tocopherol (Vitamin E)	$C_{29}H_{50}O_2$ (416.68)

46	D-Mannitol, 1,2:3,4:5, 6-tris-O-(1-methylethylidene)-	$C_{12}H_{22}O_6$ (302.36)
47	Docosanal	$C_{22}H_{44}O$ (326.61)
48	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$ (212.41)
49	Dodecanoic acid (Lauric acid)	$C_{12}H_{24}O_2$ (201.31)
50	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$ (268.43)
51	Eicosanal-	$C_{20}H_{40}O$ (296.53)
52	Eicosane	$C_{20}H_{42}$ (282.54)
53	Eicosane, 2-methyl-	$C_{21}H_{44}$ (296.57)
54	Eicosyl octyl ether	$C_{28}H_{58}O$ (410.75)
55	Ethanol, 2-(dodecyloxy)-	$C_{14}H_{30}O_2$ (230.39)
56	Fumaric acid, cis-hex-3-enyl tetradecyl ester	$C_{24}H_{42}O_4$ (280.36)
57	Heptacos-1-ene	$C_{27}H_{54}$ (378.71)
58	Heptadecane	$C_{17}H_{36}$ (240.47)
59	Heptadecane, 2,6,10, 15-tetramethyl-	$C_{21}H_{44}$ (296.57)
60	Hexadecanal	$C_{16}H_{32}O$ (256.42)
61	Hexadecane (Cetane)	$C_{16}H_{34}$ (226.41)
62	Hexadecane, 2,6,11, 15-tetramethyl-	$C_{20}H_{42}$ (282.5)
63	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ (270.45)
64	l-(+)-Ascorbic acid 2, 6-dihexadecanoate (vitamin C)	$C_{38}H_{68}O_8$ (652.9)
65	Neophytadiene	$C_{20}H_{38}$ (278.5)
66	N-Heptanoyl-L-homoserine lactone	$C_{11}H_{19}NO_3$ (213.3)
67	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$ (256.42)
68	n-Nonadecanol-1	$C_{19}H_{40}O$ (284.5)
69	Nonacos-1-ene	$C_{29}H_{52}$ (406.8)
70	Nonadecane, 2-methyl-	$C_{20}H_{42}$ (282.5)
71	Nonadecyl Pentafluoropropionate	$C_{22}H_{39}F_5O_2$ (430.5)
72	Nonadecyl trifluoroacetate	$C_{21}H_{39}F_3O_2$ (380.52)
73	Nonyl tetradecyl ether	$C_{23}H_{48}O$ (340.6)
74	n-Tetracosanol-1	$C_{24}H_{50}O$ (354.65)
75	Octacosane, 2-methyl-	$C_{29}H_{60}$ (408.8)
76	Octadecanal (Stearaldehyde)	$C_{18}H_{36}O$ (268.5)
77	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$ (284.48)
78	Octatriacontyl Pentafluoropropionate	$C_{41}H_{77}F_5O_2$ (697.0)
79	Octyl tetracosyl ether	$C_{32}H_{66}O$ (466.9)
80	Pentadecane	$C_{15}H_{32}$ (212.42)
81	Phenol, 2,4-bis(1, 1-dimethylethyl)-, phosphite (3:1)	$C_{42}H_{63}O_3P$ (646.92)
82	Phenol, 2,4-di-t-butyl-6-nitro-	$C_{14}H_{21}NO_3$ (251.32)
83	Phthalic acid, 5-methylhex-2-yl butyl ester	$C_{19}H_{28}O_4$ (320.4)
84	Phthalic acid, hept-3-yl isobutyl ester	$C_{19}H_{28}O_4$ (320.4)
85	Phthalic acid, hept-4-yl isobutyl ester (	$C_{19}H_{28}O_4$ (320.4)
86	Phytol	$C_{20}H_{40}O$ (296.53)
87	Pregnan-20-one, 5,6-epoxy-3,17-dihydroxy-16-methyl-, (3ß,5a,6a,16a)	$C_{22}H_{34}O_4$ (362.5)
88	Sebacic acid, di(2-propylpentyl) ester	$C_{26}H_{50}O_4$ (426.7)
89	Squalene	$C_{30}H_{50}$ (422.8)
90	tert-Hexadecanethiol (gold(3+) salt)	$C_{48}H_{99}AuS_3$ (969.5)
91	Tetradecane	$C_{14}H_{30}$ (198.39)
92	trans-13-Octadecenoic acid, methyl ester (Oleic acid)	$C_{18}H_{34}O_2$ (282.5)
93	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}C_{13}O_2$ (387.8)

season. Whereas, 33 phytochemicals numbered 4, 8, 10, 11, 12, 14, 18, 22, 28, 29, 32, 33, 34, 38, 39, 41, 42, 43, 44, 50, 54, 56, 59, 66, 67, 71, 75, 78, 83, 84, 86, 87 and 90 were only detected in winter. Similarly, 17 phytochemicals numbered 1, 2, 3, 6, 15, 16, 21, 25, 31, 37, 48, 51, 58, 68, 72, 79 and 91 were unique in summer (Table 3).

Comparison of per cent peak area (Table 3) revealed that l-(+)-Ascorbic acid 2,6-dihexadecanoate; Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1); dl-a-Tocopherol; 2,4-Di-tert-butylphenol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Neophytadiene; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-; 2-Dimethyl(ethenyl)silyloxytetradecane; Sebacic acid, di(2-propylpentyl) ester; 2,3-Dimethyl-3-pyrazolin-5-one; 2-Propenoic acid, 2-(acetylamino)-; Nonadecane, 2-methyl-; 4-Heptanol, 2,6-dimethyl-4-(1-methylethyl)-; 1-Hexadecanol; 9-Nonadecene; Octadecanoic acid; Eicosane, 2-methyl-; 10-Heneicosene (c,t); 2-Pentadecanone, 6,10,14-trimethyl-; D-Mannitol, 1,2:3,4:5,6-tris-O-(1-methylethylidene)- were found in higher concentration (decreasing order) in winter and summer, respectively.

Paritala *et al.* (2015) reported that different members of Meliaceae family such as *A. indica* and *M. dubia* possess a vast array of biologically active compounds which are chemically diverse and structurally complex. Earlier, Parmar *et al.* (2019) reported 18 different types of phytochemicals in *M. dubia* leaf through GC–MS analysis. Murugesan *et al.* (2013) reported 42 phytochemicals from the *M. dubia* leaf. Jahirhussain *et al.* (2015) identified 33 phytochemicals as constituents with more than one peak area for 7 compounds from ethanolic leaf extract of *M. composita*. Mudhafar *et al*. (2020) reported 22 phytochemical compounds from *M. dubia* leaves using GCMS analysis. Our GC-MS analysis revealed greater number of phytochemicals as compared to previous reports on this species. The results deduced that there was variation in phytochemical compound occurrence among provenances and seasons. Synthesis of chemical compounds within plant system happens in response to external environmental stimuli caused by edapho-climatic stresses. It could be also be due to biotic stimuli (insect pest attack) to which plant develop defence and compounds synthesized could be beneficial for human beings and wild and domestic animals feeding on it (Tekli´c *et al*., 2021; Sukhadiya *et al*., 2022).

Corroboration of chemical compounds detected in our study with available literature revealed that they occur in other species of same family (Balasubramanian *et al*., 2014; Kumar *et al*., 2018) and have one or the other beneficial biological activities. The most common are anti-bacterial, anti-inflammatory agent, antioxidant, insecticidal, analgesic, antipyretic, antiarthritic, anticancer, antihistaminic, antieczemic, antiandrogenic, hypo-cholesterolemic, antiviral anxiolytic activity *etc*. Many of them are used to cure gum disease and skin infections and other ailments (Wang and Huang, 2012; Hamid *et al.*, 2016; Ruthisha *et al*., 2017; Gravel *et al*., 2017; Swamy *et al.*, 2017; Amudha *et al*., 2018; Elgorban *et al*., 2019; Zhao *et al*., 2020; Bratty *et al*., 2020; Chikezie *et al*., 2020; Subramanian *et al*., 2020; Prodhan and Mridu, 2021; Huang *et al*., 2021; Sethuraman and Ramachandran 2022; Moussaoui *et al*., 2022). Thus, *M. dubia* leaf volatile phytochemicals are biologically active compounds and the leaf fodder could beneficial as top feed for livestock.

The presence of volatile compounds in *M. dubia* leaf could be source antioxidant, anti-inflammatory, and antibacterial activity *etc*. (Diaz-Sanchez *et al*., 2015; Kumar and Goel, 2019; Wong *et al*., 2020). And its feeding to small ruminants and livestock may have positive effect toward animals in terms of favored growth performance, production quality, and enhanced endogenous antioxidant systems, possibly by directly affecting specific molecular targets or/and indirectly as stabilized conjugates affecting the metabolic pathways (Hamaker *et al*., 2009; Jorgensen *et al*., 2010; Wong *et al*., 2020). Alkaloids have antibacterial activity which could be beneficial to livestock. Achi *et al*. (2017) reported that flavonoids (1.36%) present in *Ficus capensis* leaves possess medicinal benefits which includes antioxidant and anti-inflammatory activity. Saponins of plant origins are important dietary supplements and exhibit antimicrobial activities and protect plants from microbial pathogens. Tree leaves show high digestibility values associated with low TTC levels (Forwood and Owensby, 1985). Total tannin content range of *M. dubia* leaf in present study fall within the values (0.89 to 30.43%) reported in other top feed species (Niranjan *et al*., 2007; Datt *et al*., 2008; Wankhede and Jain, 2011; Achi *et al*., 2017). TTC values of *M. dubia* leaf also fall within the range advocated suitable in livestock feed (Devasena and Adilaxmamma, 2016).

4. CONCLUSION

Gas-chromatography mass-spectrometry (GC-MS) analysis of *M. dubia* leaf revealed variation in volatile chemical compounds across provenances and seasons. Maximum 55 and 35 compounds were detected in winter and summer season, respectively. Study revealed significant variation in total alkaloid, flavonoids, phenolic, saponin, tannin and total

Table 3. Provenance and seasonal variation in peak area (%) of *M. dubia* leaf fodder volatile phytochemicals (Please refer table 2 corresponding s.no. for phytochemical name)

Phytochemical*	Peak area (%)															
	S_1 (Winter)								S_2 (Summer)							
	Provenances								Provenances							
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.20	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.66
3	-	-	-	-	-	-	-	-	-	-	-	-	1.76	-	-	-
4	-	0.74	-	-	-	-	0.89	-	-	-	-	-	-	-	-	-
5	-	-	-	-	3.92	3.60	-	2.31	3.35	2.54	3.85	-	-	2.79	-	-
6	-	-	-	-	-	-	-	-	-	-	-	0.39	-	-	-	-
7	-	2.37	-	-	-	-	0.97	-	-	0.56	-	-	-	-	-	1.37
8	-	1.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	1.36	-	-	-	-	-	-	-	-	-	-	-	-	-	0.90
10	-	0.44	-	-	-	-	1.42	-	-	-	-	-	-	-	-	-
11	1.11	-	1.13	-	-	0.93	-	-	-	-	-	-	-	-	-	-
12	3.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	4.96	-	3.61	3.79	3.94	3.87	-	2.60	3.44	2.06	4.20	4.88	-	3.19	3.04	-
14	-	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	2.02	-	-
16	-	-	-	-	-	-	-	-	-	1.47	-	3.67	-	1.77	2.60	-
17	-	3.52	4.16	5.49	4.29	4.04	3.63	2.00	2.90	2.73	-	-	3.10	-	3.61	2.01
18	-	-	1.30	-	-	-	-	1.02	-	-	-	-	-	-	-	-
19	1.05	10.60	2.63	1.85	-	0.88	12.79	7.49	1.64	6.57	1.92	-	-	0.82	-	10.11
20	-	-	-	8.42	7.28	6.07	7.65	0.84	2.05	5.95	1.20	-	6.88	1.12	-	4.07
21	-	-	-	-	-	-	-	-	-	-	3.48	6.79	-	-	-	-
22	6.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	3.62	0.88	-	-	1.81	1.93	1.22	1.14	3.96	2.86	2.29	2.48	1.24	1.22	-	2.02
24	4.23	1.56	5.21	-	2.33	-	-	4.50	-	-	-	2.68	-	3.96	6.41	-
25	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	-	-
26	8.61	6.60	11.02	9.88	9.47	10.95	7.02	9.89	15.18	13.07	15.58	14.58	6.68	10.36	8.89	11.09
27	-	-	-	-	-	-	0.96	-	-	0.74	-	-	-	-	-	0.82
28	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-

29	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	5.00	-	-	-	2.35	-	-	5.24	-	-	-	-	-	-	1.21
31	-	-	-	-	-	-	-	-	-	-	2.32	3.33	-	-	-	-
32	3.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	0.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	4.03	-	3.27	-	-	-	-	-	-	-	-	-	-
35	-	4.38	-	-	-	-	5.25	8.92	4.46	-	-	-	8.26	3.59	5.43	4.67
36	4.84	-	4.49	4.34	-	-	-	-	-	-	-	4.30	4.51	-	4.30	-
37	-	-	-	-	-	-	-	-	-	-	-	-	-	1.13	-	-
38	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	-	0.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	0.54	-	-	-	-	0.69	0.58	-	1.05	-	-	0.69	-	3.09	0.48
41	0.26	1.44	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	-	1.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	-	-	-	-	-	-	2.01	-	-	-	-	-	-	-	-	-
44	4.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	2.20	3.33	7.74	4.50	3.52	1.53	6.51	13.45	1.47	6.51	9.68	5.25	10.21	6.74	8.08	8.33
46	-	3.16	-	-	-	-	2.35	-	-	1.59	-	-	-	-	-	2.86
47	-	-	-	-	0.92	-	-	-	-	0.57	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	1.09	-	-	-	-	-	-	-
49	-	1.77	2.18	2.48	1.89	1.03	3.10	2.62	1.42	1.81	1.24	2.57	3.54	1.29	2.26	1.98
50	-	-	2.94	-	-	-	-	-	-	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-	-	-	-	-	1.34	0.98	1.04	-	-
52	2.69	-	0.89	1.60	-	-	-	-	-	-	0.87	0.97	-	0.67	-	-
53	4.11	-	2.73	2.21	2.08	1.79	-	0.66	1.78	1.08	1.85	2.61	2.46	1.41	2.49	-
54	-	1.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	-	-	-	-	2.50	2.36	0.44	1.58	2.28	0.96	2.42	1.93	-	-	-	-
56	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	-	2.24	-	-	-	-	2.19	-	-	-	-	-	-	-	-	1.85
58	-	-	-	-	-	-	-	-	1.97	0.76	-	-	-	-	-	-
59	2.30	0.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	0.50	-	-	-	-	0.53	-	-	0.47	-	-
61	-	0.25	1.76	-	2.42	1.90	-	0.65	1.50	0.78	1.41	1.85	1.77	1.19	1.50	-

62	2.72	0.61	1.59	2.13	1.94	-	-	-	3.71	-	-	-	-	1.40	1.78	-
63	-	1.89	-	-	-	-	1.99	-	-	3.20	-	-	-	-	-	1.76
64	-	7.34	8.22	8.93	11.35	7.46	18.14	16.57	9.42	14.12	10.95	13.05	17.45	10.14	11.91	16.71
65	9.87	4.41	7.85	-	7.89	5.85	4.61	6.77	8.44	-	8.14	6.04	4.82	5.40	5.81	-
66	-	-	-	-	-	-	-	0.55	-	-	-	-	-	-	-	-
67	1.74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.08	-
69	-	-	2.68	2.97	-	2.60	-	1.31	2.48	1.83	2.71	2.98	-	2.32	3.12	-
70	5.17	0.52	5.21	4.96	-	-	-	-	0.91	-	4.94	-	3.88	3.68	-	-
71	-	1.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.38
73	-	-	-	-	-	-	0.61	-	-	-	-	-	-	-	-	0.47
74	-	1.26	-	-	-	-	-	-	-	-	-	-	2.87	-	-	-
75	-	-	-	5.32	-	4.61	-	-	-	-	-	-	-	-	-	-
76	2.82	-	2.72	3.02	2.69	2.54	-	0.98	2.44	1.44	2.62	2.96	2.73	2.26	3.25	-
77	-	2.92	-	-	-	-	4.23	2.64	-	2.49	-	-	-	1.02	1.29	3.47
78	-	0.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.19
80	-	-	-	-	-	0.73	-	-	-	-	0.96	-	-	-	-	-
81	-	-	-	-	13.83	10.59	-	-	-	-	-	-	-	14.43	-	7.26
82	-	1.90	-	-	-	-	2.15	-	-	1.20	-	-	-	-	-	1.76
83	-	-	-	-	-	2.68	-	-	-	-	-	-	-	-	-	-
84	-	0.70	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	-	-	-	-	-	-	0.72	-	-	0.69	-	-	1.41	-	0.81	-
86	0.36	0.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	-	0.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	-	-	5.28	6.07	7.83	5.52	-	2.77	6.56	4.46	6.06	7.16	8.89	5.09	7.99	-
89	0.59	-	1.04	1.02	0.68	0.74	-	0.68	0.84	-	-	1.08	-	-	-	-
90	0.44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	-	-	-	-	2.22	-	-	-	-	-
92	-	1.71	1.88	1.86	-	-	-	1.22	1.53	2.04	-	-	-	-	2.22	1.68
93	-	2.36	-	-	-	-	-	-	-	0.66	-	-	-	-	-	-

P_1-Sagai-1, P_2-Sagai-2, P_3-Nana Pondha, P_4-Kaprada, P_5-Waghai, P_6-Chichinagawtha, P_7-Mahal, P_8-Ahwa

Table 4. Seasonal variation in peak area (%) of common volatile phytochemicals of *M. dubia* leaf fodder

S.No.	Phytochemical	Max. peak area (%)	
		S_1 (Winter)	S_2 (Summer)
1.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	18.14	17.45
2.	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	13.83	14.43
3.	dl-a-Tocopherol	13.45	10.21
4.	2,4-Di-tert-butylphenol	12.79	10.11
5.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	11.02	15.58
6.	Neophytadiene	9.87	8.44
7.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	8.92	8.26
8.	2-Dimethyl(ethenyl)silyloxytetradecane	8.42	6.88
9.	Sebacic acid, di(2-propylpentyl) ester	7.83	8.89
10.	2,3-Dimethyl-3-pyrazolin-5-one	5.49	3.61
11.	2-Propenoic acid, 2-(acetylamino)-	5.21	6.41
12.	Nonadecane, 2-methyl-	5.21	4.94
13.	4-Heptanol, 2,6-dimethyl-4-(1-methylethyl)-	5.00	5.24
14.	1-Hexadecanol	4.96	4.88
15.	9-Nonadecene	4.84	4.51
16.	Octadecanoic acid	4.23	3.47
17.	Eicosane, 2-methyl-	4.11	2.61
18.	10-Heneicosene (c,t)	3.92	3.85
19.	2-Pentadecanone, 6,10,14-trimethyl-	3.62	3.96
20.	D-Mannitol, 1,2:3,4:5,6-tris-O-(1-methylethylidene)-	3.16	2.86
21.	Dodecanoic acid	3.10	3.54
22.	Octadecanal	3.02	3.25
23.	Nonacos-1-ene	2.97	3.12
24.	Hexadecane, 2,6,11,15-tetramethyl-	2.72	3.71
25.	Eicosane	2.69	0.97
26.	Ethanol, 2-(dodecyloxy)-	2.50	2.42
27.	Hexadecane	2.42	1.85
28.	1-Decanol, 2-hexyl-	2.37	1.37
29.	Trichloroacetic acid, hexadecyl ester	2.36	0.66
30.	Heptacos-1-ene	2.24	1.85
31.	Phenol, 2,4-di-t-butyl-6-nitro-	2.15	1.76
32.	Hexadecanoic acid, methyl ester	1.99	3.20
33.	trans-13-Octadecenoic acid, methyl ester	1.88	2.22
34.	1-Dodecanol, 2-hexyl-	1.36	0.90
35.	n-Tetracosanol-1	1.26	2.87
36.	Squalene	1.04	1.08
37.	3-Octenoic acid, 4-methyl-2-pentyl ester	0.96	0.82
38.	Docosanal	0.92	0.57
39.	Pentadecane	0.73	0.96
40.	Phthalic acid, hept-4-yl isobutyl ester	0.72	1.41
41.	Bis(2-ethylhexyl) phthalate	0.69	3.09
42.	Nonyl tetradecyl ether	0.61	0.47
43.	Hexadecanal	0.50	0.53

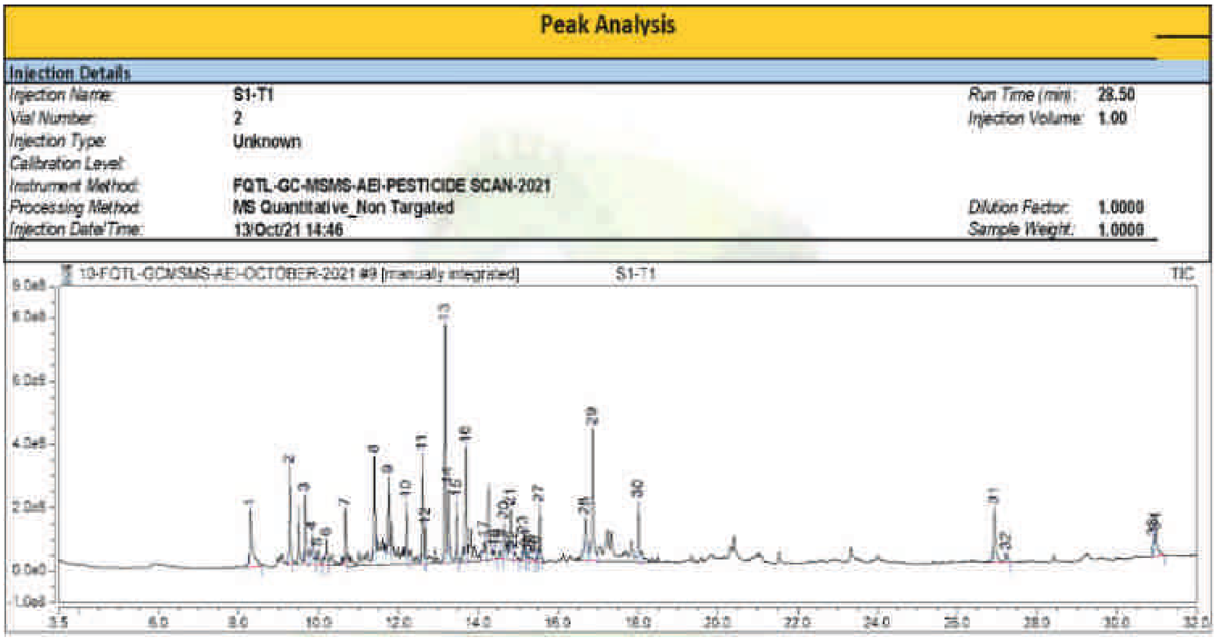

Fig. 1. Chromatogram showing *M. dubia* leaf volatile phytochemical compounds detected in winter season sample of provenance 1 (Sagai-

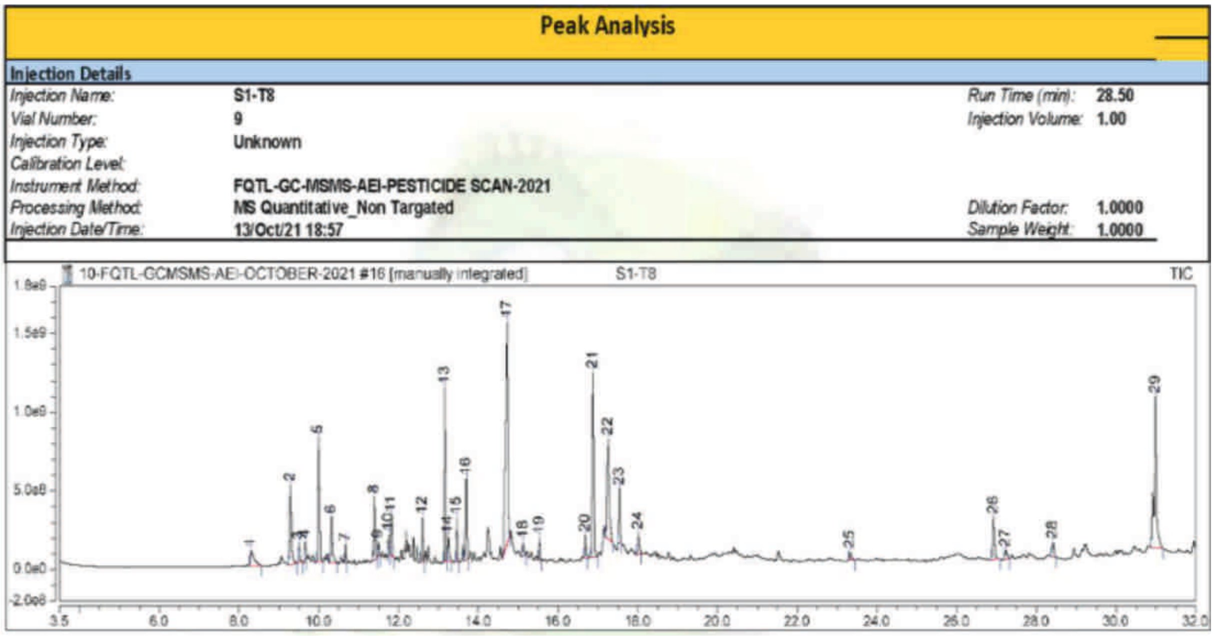

Fig. 2. Chromatogram showing *M. dubia* leaf volatile phytochemical compounds detected in winter season sample of provenance 8 (Ahwa)

terpenoids content across selected provenances and sampling seasons *i.e.* winter and summer. Corroboration of chemical compounds with available literature revealed that similar compounds occur in other species of same as well others families and have one or the other beneficial biological activities. The presence of volatile and non-volatile (secondary metabolites) compounds in *M. dubia* leaf could be source of antioxidant, anti-inflammatory, and antibacterial activity *etc.* and feeding leaves to small ruminants and livestock may have antioxidant and other health benefits to animals in terms of favored growth performance, production quality, and enhanced endogenous antioxidant systems.

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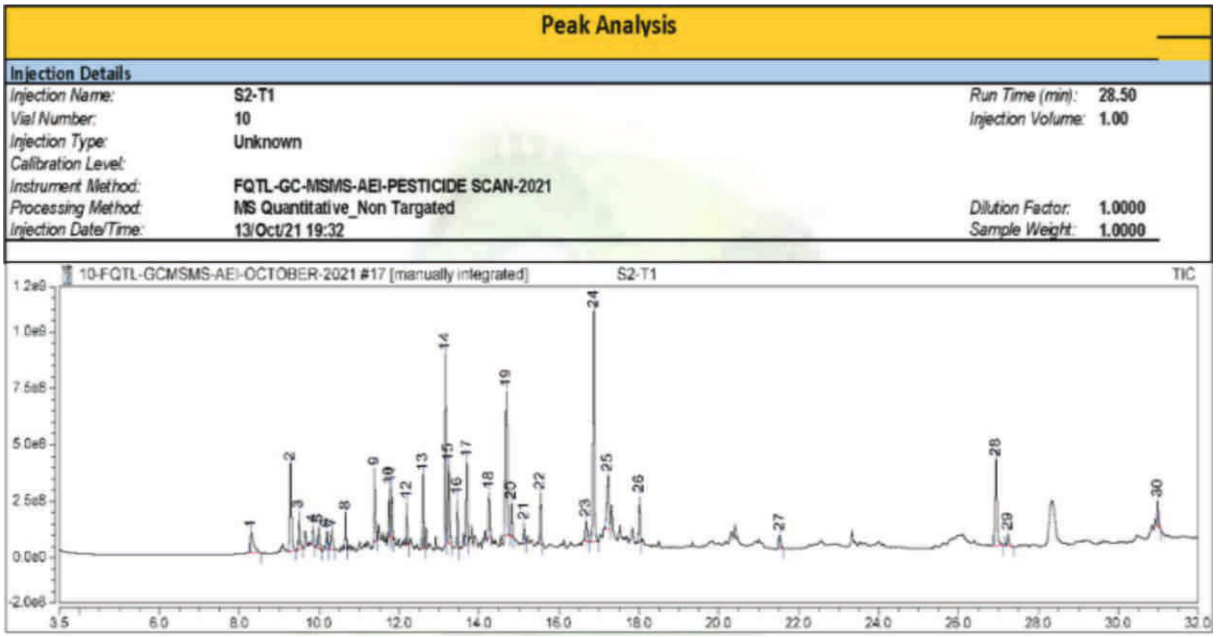

Fig. 3. Chromatogram showing *M. dubia* leaf volatile phytochemical compounds detected in summer season sample of provenance 1 (Sagai-1)

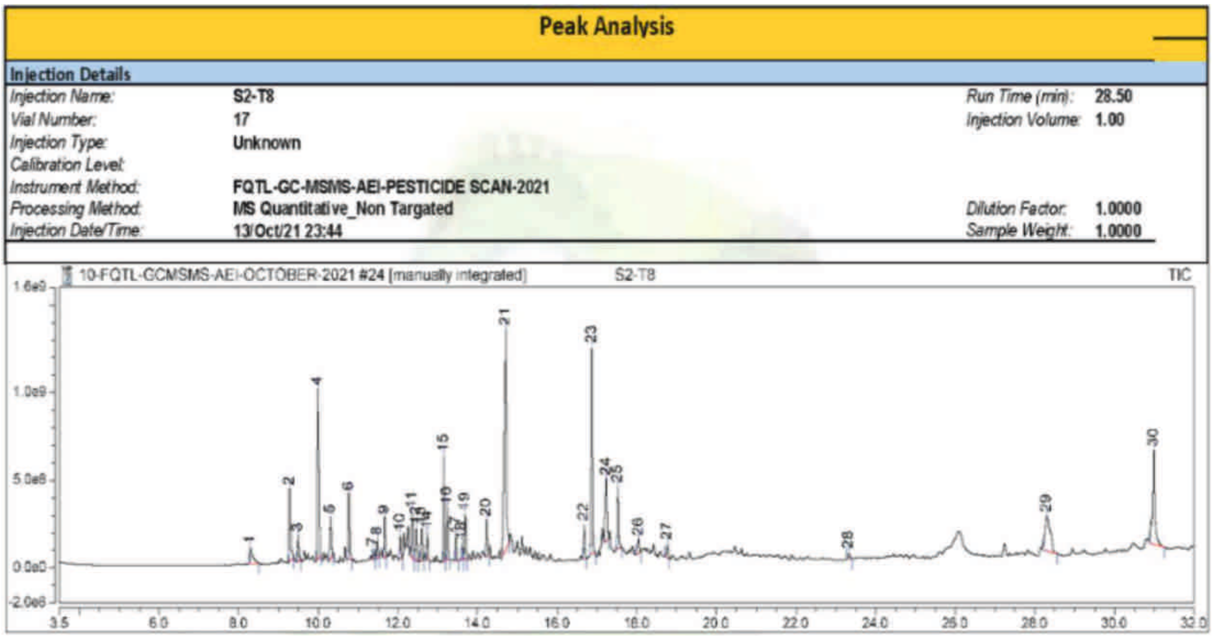

Fig. 4. Chromatogram showing *M. dubia* leaf volatile phytochemical compounds detected in summer season sample of provenance 8 (Ahwa)

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